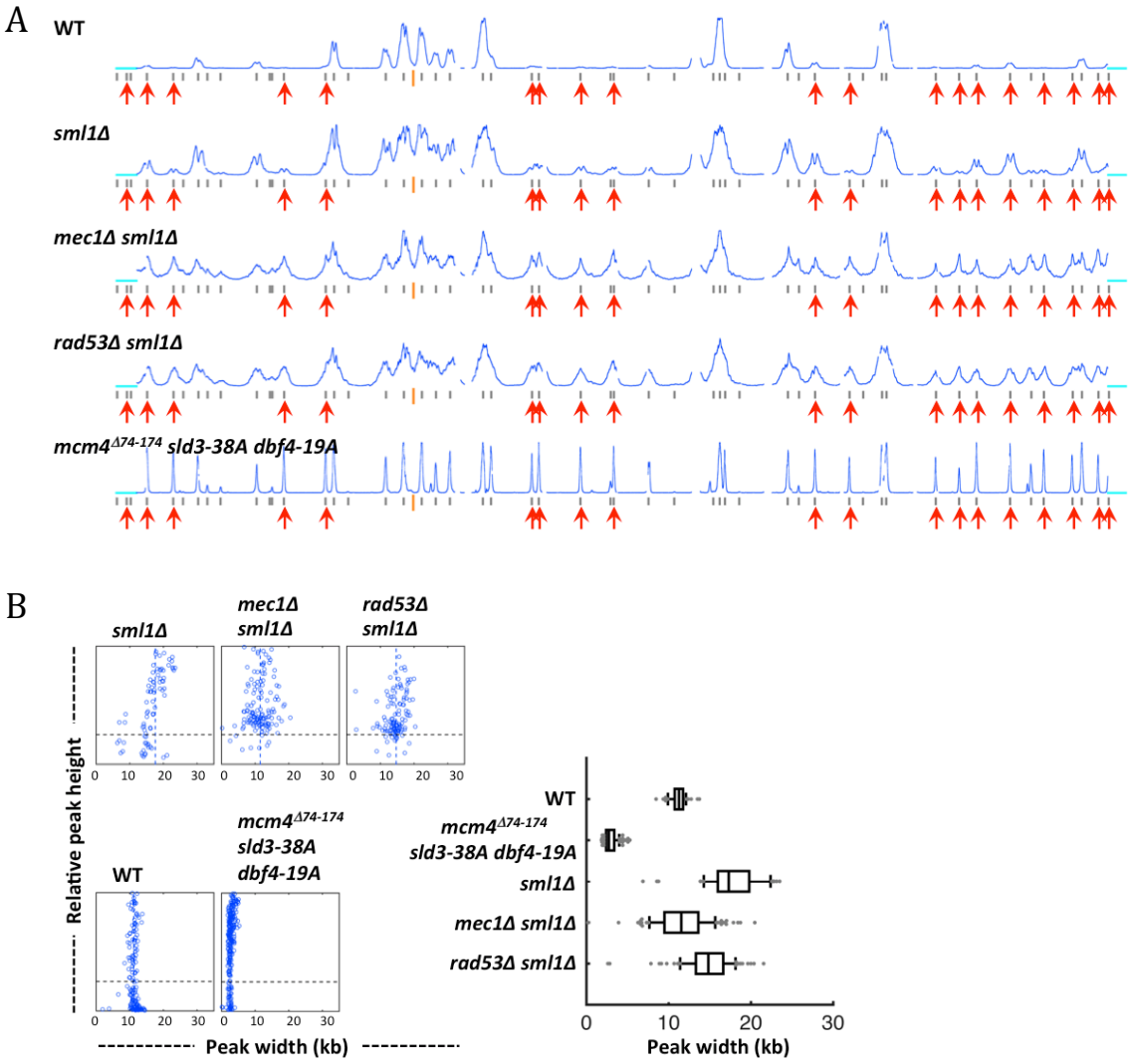

Figure S1. Late origin firing in HU in the absence of checkpoint signaling kinase. Yeast cells were synchronized in G1 phase and released in to YPD containing 0.2 M HU and 0.5 mM EdU for 90 min. (A) Replication profiles of chromosome IV for WT, *sml1Δ*, *mec1Δ sml1Δ*, *rad53Δ sml1Δ* and the *mcm4*$^{\Delta 74\text{-}174}$ *sld3-38A dbf4-19A* triple mutant cells. The cyan lines mark the 30 kb region at the end of the chromosome that was masked. See main text for detail. (B) Distribution of fork progression from origins shown as individual width-height plots and box graph, excluding peaks with heights smaller than 30 % of the maximal height scale. The analysis was done using data with 30 kb at each end of each chromosome masked.

Figure S2. Removing the Mcm4 proximal NSD allows advanced firing of late origins in an unperturbed S phase. (Continued on next page)

C

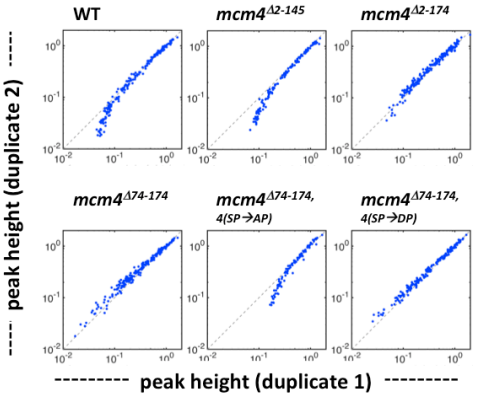

Figure S2. Removing the Mcm4 proximal NSD allows advanced firing of late origins in an unperturbed S phase. (Continuing from previous page) Experiment were performed as in Fig. 6A in duplicates. (A and B) Replication profiles of chromosome IV for WT and Mcm4 NSD mutants from duplicate 1 and 2, respectively. (C) Plots of peak height comparison for the entire genome for duplicate 1 versus duplicate 2 for each strain. Peak heights for each sample were scaled so that the 90% quantile is equal to 1.0.

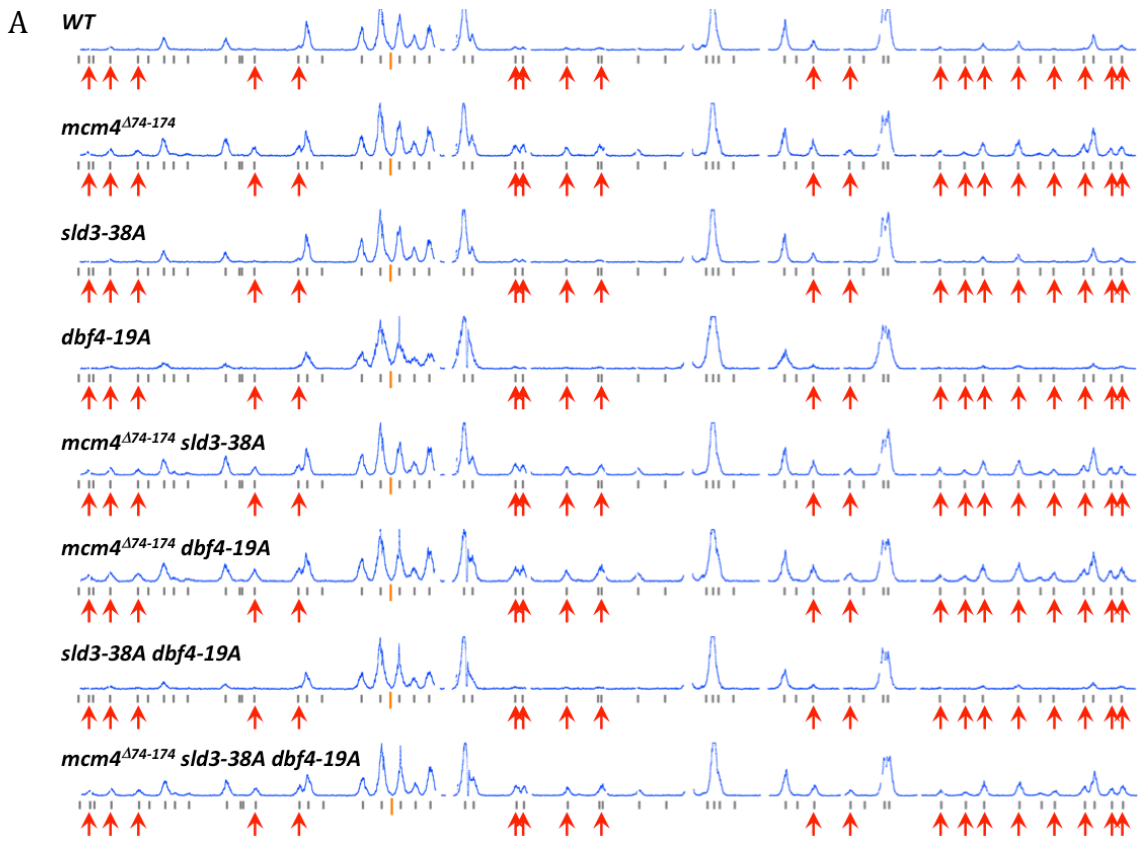

Figure S3. Checkpoint resistant mutations of *SLD3* and *DBF4* do not affect timing of late origin firing in an unperturbed S phase. (Continued on next page)

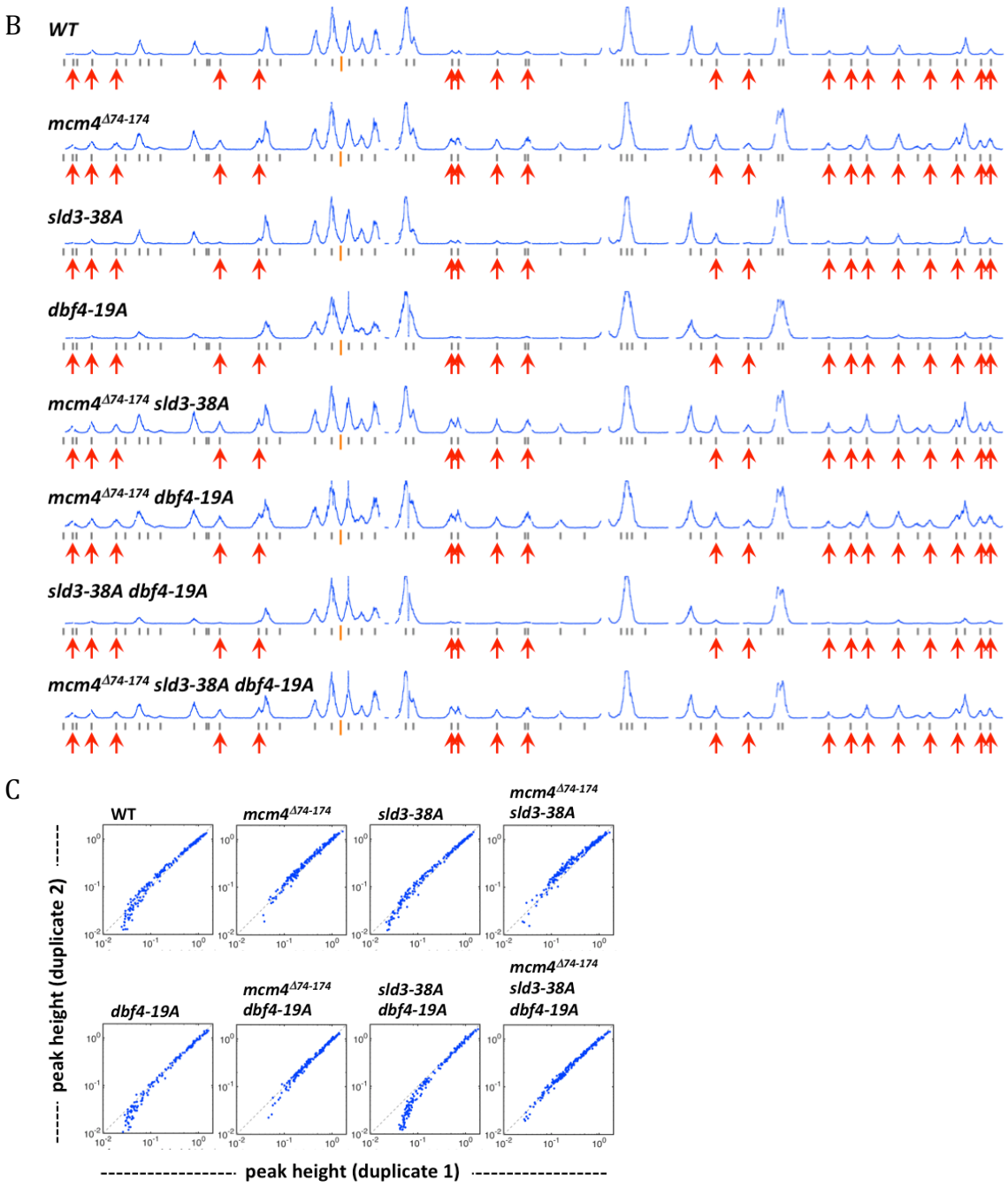

Figure S3. Checkpoint resistant mutations of *SLD3* and *DBF4* do not affect timing of late origin firing in an unperturbed S phase. (Continuing from previous page) Experiment were performed as in Fig. 7A in duplicates. (A and B) Replication profiles of chromosome IV for WT and Mcm4 NSD mutants from duplicate 1 and 2, respectively. (C) Plots of peak height comparison for the entire genome for duplicate 1 versus duplicate 2 for each strain. Peak heights for each sample were scaled so that the 90% quantile is equal to 1.0.

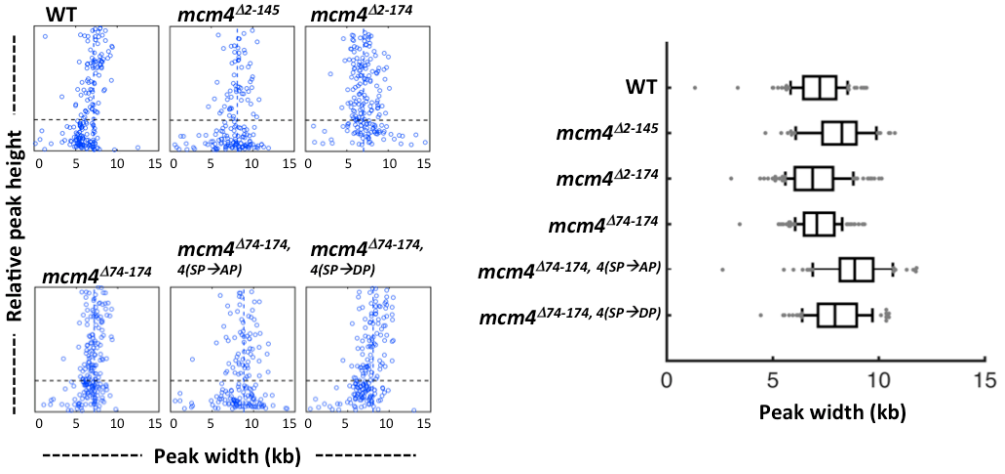

Figure S4. Distribution of fork progression from origins shown as individual width-height plots and a box graph, which excludes peaks with heights smaller than 30 % of the maximal height scale. Analysis was done using the same data presented in Fig. 6A.

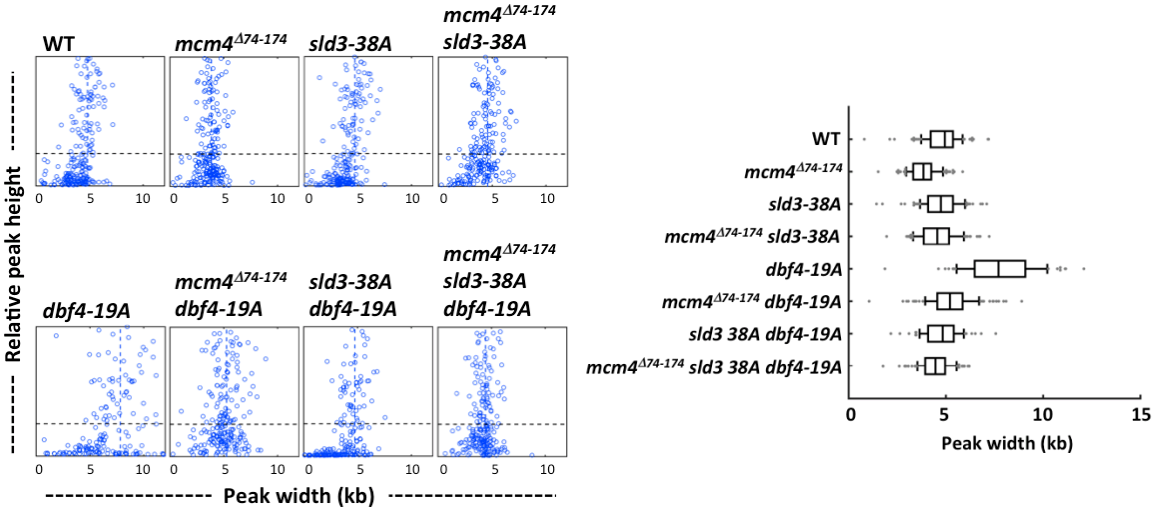

Figure S5. Distribution of fork progression from origins shown as individual width-height plots and a box graph, which excludes peaks with heights smaller than 30 % of the maximal height scale. Analysis was done using the same data presented in Fig. 7A.